# PARASITE FAUNA OF AUSTRALIAN MARINE OLIGOCHAETES

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A survey of 7,200 marine oligochaetes from Queensland (Moreton Bay, Brisbane, Heron Island and Lizard Island), New South Wales (Georges River, Sydney) and the Northern Territory (Darwin Harbour) revealed infections by 5 major parasite groups. Tubificid oligochaetes of the Limnodriloidinae, Phallodrilinae and Rhyacodrilinae were host to: 10 actinosporeans (Myxozoa) namely *Sphaeractinomyxon ersei*, *S. leptocapsula*, *Endoeapsa rosulata*, *E. stepheni*, Endocapsa type 1 nov., *Tetraspora diseoidea*, *T. rotundum*, Triactinomyxon of Roubal et al., 1997, Triactinomyxon type 1 nov. and Triactinomyxon type 2 nov.; an aseptate eugregarine (Apicomplexa) *Oligochaetocystis* sp.; an astomate ciliate (Ciliophora); a peritrichous ciliate *Seyphidia* sp. (Ciliophora); mermithid nematodes (Nematoda); a haplosporidian (Haplosporidia); and a coccidian (Apicomplexa). A single enchytraeid specimen, *Grania* sp., harboured astomate ciliates. ❐ *Australia, actinosporeans, marine oligochaetes, parasites, protozoans.*

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The literature suggests that parasites of marine oligochaetes are common (Giere & Pfannkuche, 1982; Raftos & Cooper, 1990), yet few specific examples can be found. Documented parasites belong principally to the protozoan orders Astomata (Ciliophora: Holotricha) and Gregarinida (Apicomplexa: Telosporidia) with at least 13 and 5 species described, respectively (Giere & Pfannkuche, 1982). Six actinosporeans (Myxozoa), *Sphaeractinomyxon stolci* Caullery & Mesnil, 1904, *Sphaeractinomyxon* type 1 & 2 Hallett et al., 1997a and *Aurantiactinomyxon* type 1, 2 & 3 Hallett et al., 1997a, also have been recorded. Prior to this study, no parasites had been recorded from an Australian marine oligochaete.

Freshwater oligochaetes are reported as hosts to these parasite groups as well as to cestodes (e.g. *Archigetes iowensis* and *Hunterella nodulosa*), nematodes (e.g. *Eustrongylides* sp., *Dioctophyma renale*) (Raftos & Cooper, 1990) and rotifers *Albertia* spp. (Koste, 1970; Erséus, 1976). They also are host to about 116 actinosporeans (McGeorge et al., 1997; Lom et al., 1997; Xiao & Desser, 1998a, b; El-Mansy et al., 1998b,c); some of these parasites are alternate stages in the life cycles of myxosporeans (Myxozoa) in fish (see Kent et al., 1994a; Lom et al., 1997). To date, some 24 life cycles have been elucidated and they have all involved freshwater species for which no other form of life cycle is known. No complete life cycle has been determined for any marine myxozoan. Myxosporeans are common in marine fish in Australia; this study was undertaken to determine if actinosporeans were present in Australian marine oligochaetes. Some of our early findings have been published elsewhere. These records are of the actinosporeans *Sphaeractinomyxon ersei* Hallett, O'Donoghue & Lester, 1998, *S. leptocapsula* Hallett, Erséus & Lester, 1999, *Endocapsa rosulata* Hallett, Erséus & Lester, 1999, *E. stepheni* Hallett, Erséus & Lester, 1999, *Tetraspora discoidea* Hallett & Lester, 1999, *T. rotundum* Hallett & Lester, 1999, and Triactinomyxon of Roubal et al., 1997. Here, we present a review of all parasite types so far encountered in Australian marine oligochaetes, including published records.

## MATERIALS AND METHODS

Sediment samples were collected to a depth of 15cm from the intertidal zone in Moreton Bay (27°15-25'S), and from Heron (23°27'S, 151°55'E) and Lizard (14°40'S, 145°28'E) Islands during 1995-1997 (Fig. 1). Infected oligochaetes were also obtained from Sydney, NSW (33°53'S, 151°10'E) and Darwin, NT (12°25'S, 130°51'E) (Fig. 1B). Sediment was collected in 500ml jars, each emptied into a

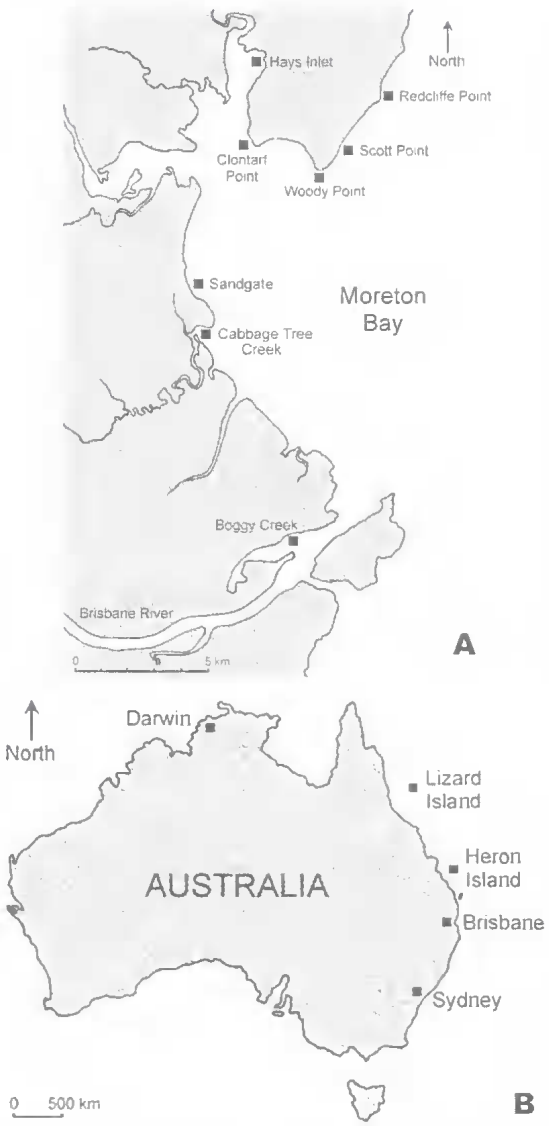



FIG. 1. A, Moreton Bay collecting localities. B, Map of Australia showing other collecting localities. Brisbane incorporates Moreton Bay.

bucket containing about 4 litres of seawater, thoroughly mixed and the supernatant poured through a 0.4mm sieve. The contents of the sieve were washed into a petri dish and the supernatant returned to the bucket. This process was repeated about 5 times per sample. The material in the petri dish was then examined under a dissection microscope and any oligochaetes present were recovered for detailed examination under a compound microscope. The worms were placed on glass slides and a drop of 25% ethanol in seawater was added to inhibit movement. Parasites (with the exception of those obtained from Lizard Island, Darwin Harbour and Sydney) were photographed, sketched and measured prior to fixation in either Bouin's fixative for 24 hours for host identification or 3% gluteraldehyde in 0.066M cacodylate buffer for 24 hours for electron microscopy. For host identification, infected oligochaetes were transferred to 70% ethanol, stained in alcoholic paracarmine, mounted whole in Canada Balsam and examined under a compound microscope. Nematodes were fixed within the oligochaete host and several were cleared and stained with chlorlactophenol and Mayer's haemotoxylin. Actinosporean descriptions follow the guidelines presented by Lom et al. (1997) except that 'germ cell' is used for 'daughter cells' and we give an additional measurement 'basal width' which is maximum width of the spore in apical view. New forms are identified in accordance with Kent et al. (1994) and Lom et al. (1997). Reference oligochaete host specimens containing parasites are lodged in the Queensland Museum (QM), Brisbane.

## RESULTS

A total of 5,200 oligochaetes were examined from Moreton Bay and a further 2,000 oligochaetes from Heron Island. The density of worms sampled in Brisbane was up to 0.8 worms per $cm^3$. Six parasitic/commensal groups were identified: actinosporeans (Myxozoa), aseptate cugregarines (Apicomplexa), astomate and peritrichous ciliates (Ciliophora), mermithid nematodes (Nematoda), haplosporidians (Haplosporidia) and coccidians (Apicomplexa) (Tables 1, 2).

### Phylum MYXOZOA Grassé, 1970
### Class MYXOSPOREA Bütschli, 1881
### ACTINOSPOREAN FORMS Kent et al., 1994

Infections by actinosporeans were detected in 196 (3.8%) of 5,200 worms from Moreton Bay, 25 (1.3%) of 2,000 worms from Heron Is. and in 8 worms from Lizard Is. One worm from Moreton Bay harboured a double infection of *Sphaeractinomyxon ersei* and *Tetraspora discoidea*. Three worms, *Duridrilus* sp.(QMG463613), *Limnodriloides* sp. (QMG463615) and *Doliodrilus diverticulatus* (QMG463614) from Darwin Harbour and an unidentified oligochaete from Georges River, Sydney, harboured unidentified actinosporeans (all fixed samples). Ten actinosporeans belonging to 4 collective groups were found in marine oligochaetes (Table 1).

TABLE 1. Actinosporeans from Australian marine oligochaetes.

| Actinosporean | No. Infected Hosts | Host | Site |
|---|---|---|---|
| *Sphaeractinomyxon ersei* | 2 | *Doliodrilus diverticulatus* | Moreton Bay |
| | 1 | *Limnodriloides* cf. *victoriensis* | Moreton Bay |
| | 44 | Tubificidae sp./spp. | Moreton Bay |
| | 2 | Tubificidae sp./spp. | Heron Island |
| | 1 | *Thalassodrilides* cf. *gurwitschi* | Lizard Island |
| | 2 | *Limnodriloides lateroporus* | Lizard Island |
| | 1 | *Bathydrilus* sp. | Lizard Island |
| *Sphaeractinomyxon* spp. | 24 | Tubificidae sp./spp. | Moreton Bay |
| *S. leptocapsula* | 2 | *Heronidrilus* sp. | Lizard Island |
| *Endocapsa rosulata* | 3 | *Heterodrilus* cf. *keenani* | Heron Island |
| | 1 | *Thalassodrilides* cf. *gurwitschi* | Lizard Island |
| | 1 | *Heronidrilus* sp. | Lizard Island |
| | 9 | Tubificidae sp./spp. | Moreton Bay |
| *E.* cf. *rosulata* | 6 | Tubificidae sp./spp. | Heron Island |
| *E. stepheni* | 1 | *Heterodrilus* cf. *keenani* | Heron Island |
| | 2 | *Heterodrilus queenslandicus* | Heron Island |
| | 1 | Tubificidae sp. | Heron Island |
| Endocapsa type 1 | 8 | Tubificidae spp. | Moreton Bay |
| *Tetraspora discoidea* | 2 | *Doliodrilus diverticulatus* | Moreton Bay |
| | 12 | Tubificidae sp./spp. | Moreton Bay |
| *Tetraspora rotundum* | 3 | Tubificidae sp./spp. | Moreton Bay |
| *Triactinomyxon* sp. | 1 | *Limnodriloides* cf. *victoriensis* | Moreton Bay |
| | 24 | Tubificidae sp./spp. | Moreton Bay |
| Triactinomyxon type 1 | 1 | Limnodriloidinae sp. | Moreton Bay |
| Triactinomyxon type 2 | 1 | Tubificidae sp. | Moreton Bay |
| Unidentified: | | | |
| Sphaeractinomyxid | 10 | Tubificidae sp./spp. | Heron Island |
| coelomic infection | 53 | Tubificidae sp./spp. | Moreton Bay |
| intestinal infection | 12 | Tubificidae sp./spp. | Moreton Bay |
| coelomic infection | 1 | *Duridrilus* sp. | Darwin Harbour |
| coelomic infection | 1 | *Limnodriloides* sp. | Darwin Harbour |
| coelomic infection | 1 | *Doliodrilus diverticulatus* | Darwin Harbour |
| infection | 1 | Tubificidae sp. | Sydney |

## SPHAERACTINOMYXON FORMS

### **Sphaeractinomyxon ersei** Hallett, O'Donoghue & Lester, 1998 (Fig. 2A)

TYPE HOST. *Doliodrilus diverticulatus* Erséus, 1985 (Tubificidae: Limnodriloidinae).

SITE IN HOST. Immature stages located within the coelom and mature spores present in the intestinal lumen.

TYPE LOCALITY. Boggy Creek, Moreton Bay, 27°24'S, 153°09'E.

SPECIMENS LODGED. QM G462452 (#110), G462453 (#185), G462465 (#210), G463601 (LI95-4), G463602 (LI95-24/1), G463603 (LI95-24a), G463604 (LI95-24b).

DESCRIPTION. Triradially symmetrical spores packed in groups of eight in the pansporocyst. Spores triangular in apical view, diameter 17-34µm, basal width 17-33µm; ellipsoidal in side view, length 17-33µm. Polar capsules, 3, round to pyriform, centrally located, diameter (width) 3-5.5µm, length 3-7µm. Sporoplasm, rounded triangular in apical view, single binucleate, about 46 germ cells, almost fills the spore cavity.

REMARKS. *S. ersei* was detected in 47 (0.9%) of 5,200 oligochaetes examined from Moreton Bay; the number may be higher because 77 unidentified (immature) coelomic actinosporeans were also recorded (see Table 1). It also infected *Limnodriloides* cf. *victoriensis* Brinkhurst & Baker, 1979 from Boggy Creek, tubificid species from Heron Is. and *Thalassodrilides* cf. *gurwitschi* (Hrabe, 1971) (Limnodriloidinae), *Limnodriloides lateroporus* Erséus, 1997 and *Bathydrilus* sp. (immature) (Tubificidae: Phallodrilinae) from Lizard Is.

### **Sphaeractinomyxon leptocapsula** Hallett, Erséus & Lester, 1999 (Fig. 2B)

TYPE HOST. *Heronidrilus* sp. (2 immature specimens infected) (Tubificidae: Rhyacodrilinae).

TABLE 2. Parasites and commensals identified from Australian marine oligochaetes. #NR = not recorded.

| Parasite | No. Infected Hosts | Host | Site |
|---|---|---|---|
| APICOMPLEXA | | | |
| *Oligochaetocystis* sp. | 1 | Tubificidae sp. | Moreton Bay |
| Unidentified coccidian species | NR | Limnodriloidinae sp./spp. | Moreton Bay |
| CILIOPHORA | | | |
| *Radiophrya* sp. | 43+ | Tubificidae spp. | Heron Island |
| Unidentified astome | 1 | *Grania* sp. | Heron Island |
| *Scyphidia* sp. | NR | Limnodriloidinae sp./spp. | Moreton Bay |
| HAPLOSPORIDIA | | | |
| *Haplosporidium* sp. | 1 | *Heterodrilus* sp. | Heron Island |
| | 15 | Tubificidae sp./spp. | Heron Island |
| NEMATODA | | | |
| Mermithid nematode | 1 | *Heterodrilus* cf. *keenani* | Heron Island |
| | 4 | Tubificidae sp./spp. | Heron Island |

SITE IN HOST. Coelom.

TYPE LOCALITY. Intertidal sand, Lizard Is., 14°40'S, 145°28'E.

SPECIMENS LODGED. QMG462459 (LI95-16b), G462460 (LI95-16e).

DESCRIPTION. Spores triangular in apical view, diameter 20-24µm, basal width 20-23µm, length 17-22µm. Outer valve cell membrane follows contours of inner valve cell membrane. Polar capsules ~5µm long, slender, pyriform, orientated with pointed ends facing centre of spore, each positioned opposite a corner of spore about midway along spore radius, each contain polar filament with at least two turns. In side view, spore ellipsoidal to broad pyriform. Suture lines not discernible. Pansporocysts each with eight spores.

REMARKS. *S. leptocapsula* was observed on only in 2 oligochaetes from Lizard Is.

## ENDOCAPSA FORMS

### Endocapsa rosulata
Hallett, Erséus & Lester, 1999
(Fig. 2C-F)

TYPE HOST. *Heterodrilus* cf. *keenani* Erséus, 1981 (Tubificidae: Rhyacodrilinae).

SITE IN HOST. Immature stages in coelom; mature spores in intestinal lumen (Fig. 2E-F).

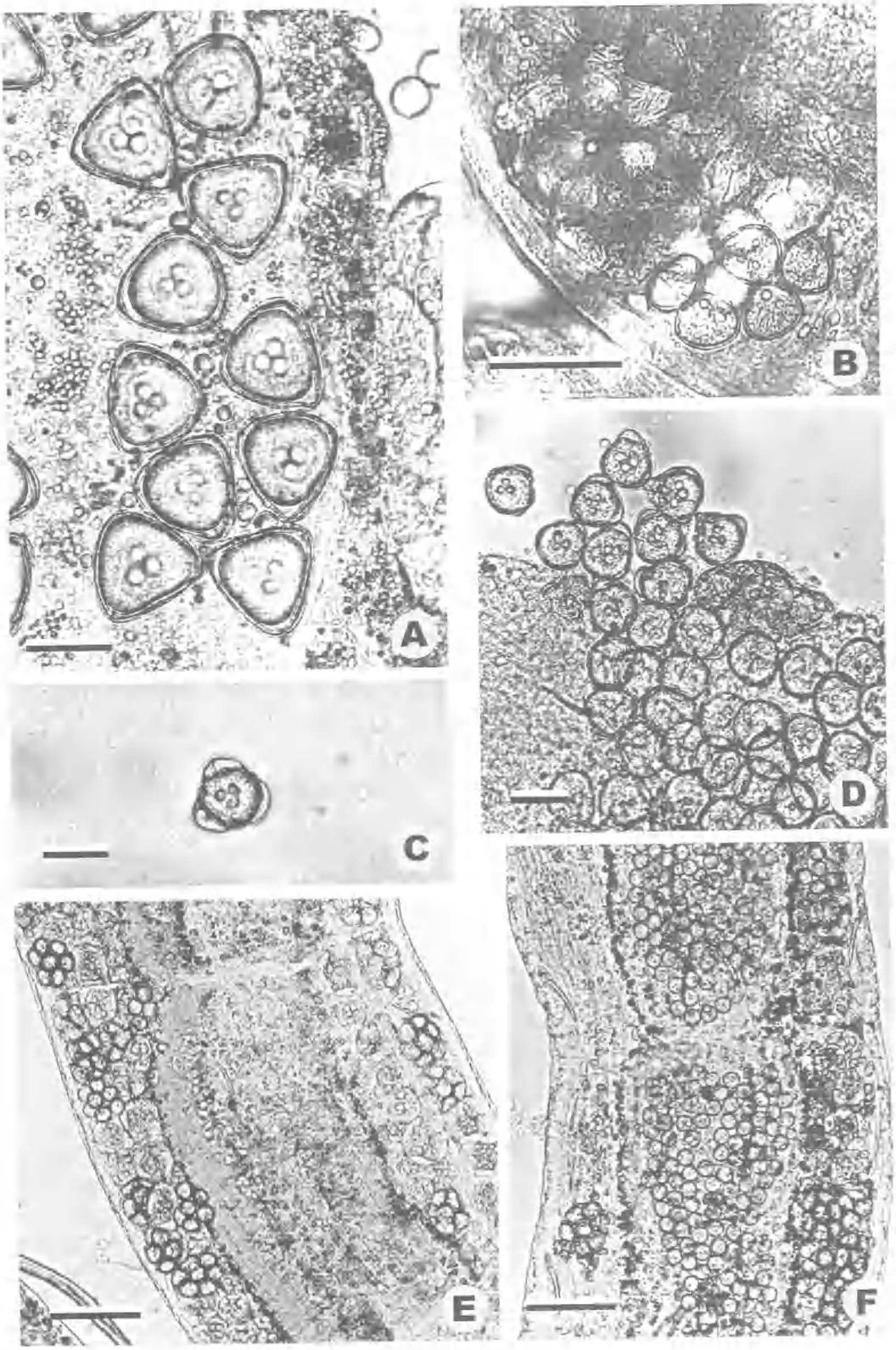
TYPE LOCALITY. Heron Is., 23°27'S, 151°55'E.

SPECIMENS LODGED. QM G462454 (H122), G462455 (H146), G463605 (LI95-24c), G463606 (LI95-16a), G462723 (#200).

DESCRIPTION. Spore diameter 25-27µm, basal width 25-28µm, length 20-23µm, resembles rosette in apical view, compressed dorsoventrally in side view (Fig. 2C); develops in groups of 8 within pansporocysts (55-75µm). Valve cells inner membrane subspherical; outer membrane follows contours of dorsal and ventral surfaces of this but exhibits swellings laterally in 3 regions equidistantly apart; inner and outer membranes close at each valve junction; suture lines prominent. Valve cell swellings formed within host, little or no further expansion on contact with seawater (Fig. 2D). Cavity diameter 19-23µm, ellipsoidal in side view. Polar capsules ellipsoidal, 4-5µm long and wide, located beneath a suture line anteriorly in, but not extruding from, spore, adjacent to remains of capsulogenic cell and perpendicular to processes, embedded in sporoplasm in side view, with polar filament with at least 3 turns. Sporoplasm granular, remaining areas of spore clear.

REMARKS. *Thalassodrilides* cf. *gurwitschi* and *Heronidrilus* sp. (immature) from Lizard Is. and tubificids from Moreton Bay also harboured this parasite. Nine tubificids of the 5,200 oligochaetes from Boggy Creek, Moreton Bay, were infected. Spores were observed only in the coelom; none were found in the gut lumen as were Heron Is. infections. Spores were smaller

FIG. 2. Actinosporeans from marine oligochaetes. A, mature *Sphaeractinomyxon ersei* spores (each with 3 polar capsules) in the intestinal lumen of a limnodriloidine oligochaete from Moreton Bay. Fresh, unstained material. Scale = 25µm; B, *Sphaeractinomyxon leptocapsula* pansporocysts with eight spores in the coelom of an oligochaete. Preserved material from Lizard Island. Scale = 50µm. C-F, *Endocapsa rosulata*, fresh unstained material. C, Moreton Bay; D-F, Heron Island. C, spore in sea water. Scale = 20µm; D, spores emerging from host. Scale = 25µm; E, developing stages of *E.* cf. *rosulata* in coelom. Scale = 150µm; F, spores of *E.* cf. *rosulata* free in intestinal lumen. Scale = 150µm.


A
B
C
D
E
F

than those from Heron Is. being 20-25µm in diameter and 25-28µm in basal width.

**Endocapsa stepheni** Hallett, Erséus & Lester, 1999

TYPE HOST. *Heterodrilus* cf. *keenani*.

SITE IN HOST. Coelom.

TYPE LOCALITY. Heron Is., 23°27'S, 151°55'E.

SPECIMENS LODGED. QM G462456 (H132), G462457 (H135), G462458 (H148).

DESCRIPTION. Spore, diameter 25-28µm, basal width 23-25µm, length ~20µm, irregularly shaped in apical view. Spore cavity diameter ~23µm. Valve cells follow shape of roughly triangular spore cavity but with a single lobe-like swelling at one corner. Sutures, detectable, from corners to middle of spore. Sporoplasm roughly triangular in apical view, almost fills spore cavity, depressed in side view where polar capsules positioned. Polar capsules round in apical view, diameter 4-5µm, pyriform in side view, length 4-5µm, centrally located in spore, close to each other, beneath suture lines. Spore appearing ellipsoidal in side view, except that valve cells form an extension at one side.

REMARKS. One *Heterodrilus* cf. *keenani*, 2 *H. queenslandicus* Jamieson, 1997 and 1 unidentified tubificid were infected with this actinosporean.

**Endocapsa type 1** nov. (Fig. 3)

HOST. Immature tubificids.

SITE IN HOST. Developing stages in peritoneum, mature spores in coelom and intestinal lumen.

LOCALITIES. Hays Inlet and Boggy Creek, Moreton Bay, 27°16'S, 153°04'E and 27°24'S, 153°09'E .

DESCRIPTION. Spores subtriangular in apical view, diameter 17-30µm (22µm, n=8), basal width 19-31µm (25µm, n=2) (Fig. 3A). Spore body (inner valve cell membrane) basically round but corner formed at 3-way valve junction (Fig. 3A, C). Valve cells, upon contact with sea water, form 3 equally-sized biconcave processes (swellings) which join at their narrowest part at each 3-way valve junction (Fig. 3A, C). Swellings frequently present prior to spore contact with seawater. Polar capsules round, oval to pyriform in side view, diameter 3-5µm (4µm, n=4), located centrally in spore, proximal to one other, each situated beneath a suture (and therefore opposite a spore corner) (Fig. 3A), embedded in anterior part of sporoplasm, do not form an apex (Fig. 3B). Spore round in side view, length 16-28µm (22µm, n=7), diameter 19-31µm (25µm, n=6) (Fig. 3B).

REMARKS. Some spores had smooth rather than pinched corners. The non-protrusive polar capsules and reduced swellings, at times present within the host, place this actinosporean in the *Endocapsa*. Two other forms of *Endocapsa* have been recorded: *E. rosulata*; and *E. stepheni*. Endocapsa type 1 differs from both these in the shape of the spore and swellings. Its valve swellings encompass fully the spore body (visible in both apical and side view), whereas those of *E. rosulata* do not, and it possesses 3 swellings whereas *E. stepheni* forms just one. The Aurantiactinomyxon types described from Hong Kong marine oligochaetes possess valve projections rather than swellings and the spores are considerably smaller, being less than 20µm (Hallett et al., 1997). The principal difference between the Neoactinomyxon types described by El-Mansy et al. (1998b) and Endocapsa type 1 is that the valve cells of the former form triangular shaped extensions whereas they are curved in the valve cells of the latter. Endocapsa type 1 was recorded from 8 tubificid specimens.

TETRASPORA FORMS

**Tetraspora discoidea** Hallett & Lester, 1999 (Fig. 4A)

TYPE HOST. *Doliodrilus diverticulatus* Erséus, 1985 (Tubificidae: Limnodriloidinae).

SITE IN HOST. Coelom.

TYPE LOCALITY. Boggy Creek, Moreton Bay, 27°24'S, 153°09'E.

SPECIMENS LODGED. QM G462461 (#104), G462462 (#114), G462463 (#96).

DESCRIPTION. Spore diameter 33-52µm, basal width 33-38µm, length 14-22µm, disc-like, almost round, valve junctions form corner in apical view, dorsoventrally compressed in side view. Valve cell processes absent. Polar capsules, 3, subspherical in apical view, each within a pyriform capsulogenic cell, situated beneath a spore suture line, opposite a spore corner, bases proximal; pyriform in side view, located midway across spore at dorsal surface of sporoplasm. Polar filament oblique, at least 7 turns. Sporoplasm, shape similar to spore, unisporal,

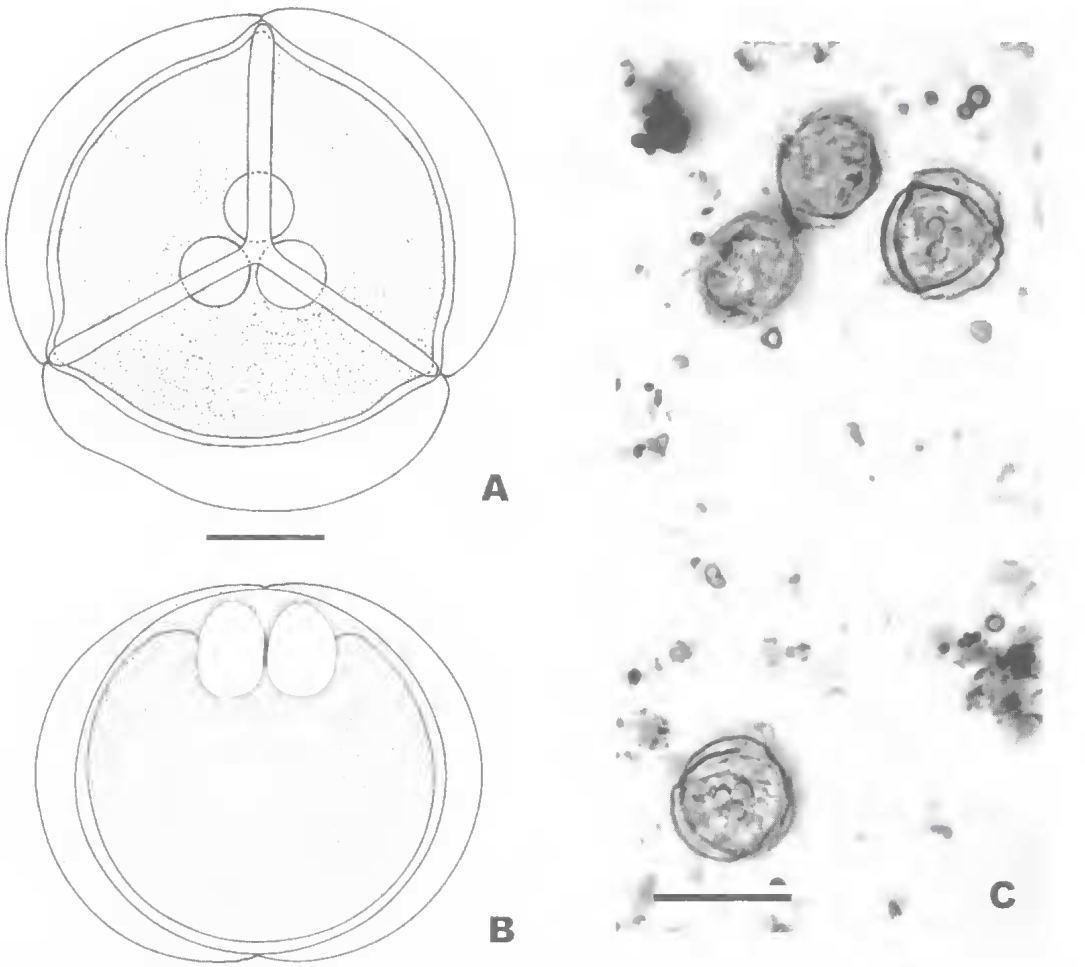



FIG. 3. Endocapsa type 1 nov. from fresh material from Moreton Bay. A, drawing of apical view and B, side view. Scale = 5µm. C, spores in seawater. Scale = 50µm.

contains at least one somatic nucleus and >100 germ cells. Pansporocysts, freely floating within host coelom, irregularly shaped, 47-70µm, each containing 4 developing spores. Spores do not alter in size or shape following release from host. Development between pansporocysts asynchronous, but within synchronous.

REMARKS. Twelve immature unidentifiable Tubificidae from Boggy Creek and 2 specimens of *D. diverticulatus* from Hays Inlet were infected with this parasite.

**Tetraspora rotundum** Hallett & Lester, 1999
(Fig. 4B)

TYPE HOST. Immature Tubificidae sp.

SITE IN HOST. Coelom.

TYPE LOCALITY. Boggy Creek, Moreton Bay, 27°24'S, 153°09'E.

SPECIMENS LODGED. QM G462464 (#113, #186).

DESCRIPTION. Triradially symmetrical spores packed in groups of eight in the pansporocyst. Spores triangular in apical view, diameter 17-34µm, basal width 17-33µm; ellipsoidal in side view, length 17-33µm. Polar capsules, three, round to pyriform, centrally located, diameter (width) 3-5.5µm, length 3-7µm. Sporoplasm, rounded triangular in apical view, single binucleate, about 46 germ cells, almost fills the spore cavity.

REMARKS. Three tubificids from Boggy Creek harboured *T. rotundum*.

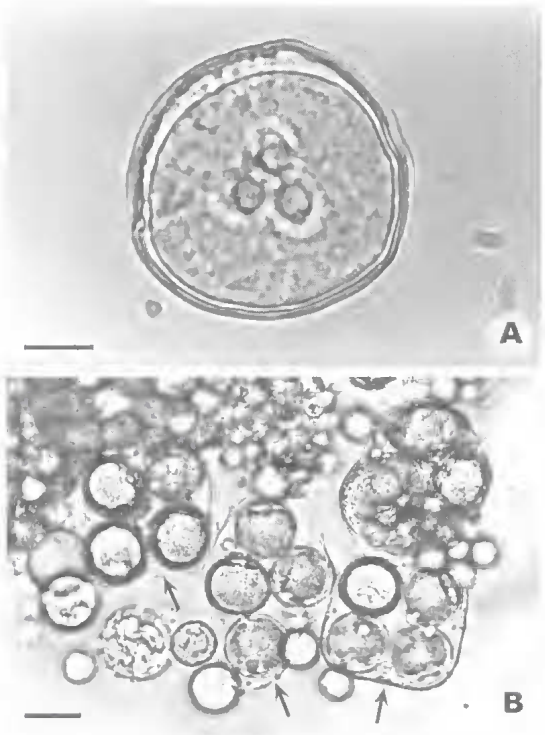



FIG. 4. *Tetraspora* spores; fresh unstained material. A, *T. discoidea* in seawater. Scale = 10μm. B, *T. rotundum* pansporocysts (arrows) with four spores. Scale = 20μm.

## TRIACTINOMYXON FORMS

### **Triactinomyxon** of Roubal et al., 1997 (Figs 5, 6)

HOST. *Limnodriloides* cf. *victoriensis*.

SITE IN HOST. Immature stages in intestinal epithelium (Fig. 6B, D) and mature spores present also in intestinal lumen.

LOCALITIES. Hays Inlet, Clontarf Point, and Boggy Creek, Moreton Bay, 27°15-25'S

DESCRIPTION (expanded from Roubal et al., 1997). Spore anchor-shaped, total length 96-142μm (125μm, n=6) (Fig. 5A). Valve cells inflate upon contact with seawater to form 3 anteriorly curved projections (caudal processes) from spore stylus at 90-100° angle (α) (Figs. 5A, 6A). Projections equal length 94-185μm (138μm, n=6) and width, at end taper to a point, equidistantly apart, arm base not wider than stylus base. Polar capsules, three, 3-5μm (4μm, n=5) long, located at anterior end of stylus, pyriform, protrude at apex, bases abut, anterior end of each at slight angle (c.45°) facing away from other polar capsules (Fig.5 inset). Stylus widens gradually from tip to base (to ~22μm). Sporoplasm, single, 17μm long, with indiscernible number of germ cells, irregularly in the stylus.

REMARKS. This Triactinomyxon most closely resembles *T. legeri* Mackinnon & Adam, 1924 and *T. ignotum* Stolc, 1899, but spore dimensions are most like *T. legeri* (style 90-140μm, arms 150μm, sporoplasm 15-20μm [Marques, 1984]). *T. legeri* and *T. ignotum* differ in size and number of germ cells in the sporoplasm (*T. legeri* = 24; *T. ignotum* = 8) and both develop in the intestinal epithelium of *Tubifex* freshwater oligochaetes. Triactinomyxon differs from *T. legeri* in its host and environment but these characters are considered insufficient to establish a new species. The number of germ cells was indeterminable by light microscopy in the marine triactinomyxon but the size of the sporoplasm suggests about 32. The orientation of the processes varied between spores emitted from a single host and were directed out, up or down; spores were always observed under a coverslip and this may have influenced the orientation of the projections. The range in spore length and arm length appears initially large but these are comparable with other species (range of 50 and 100μm respectively) (Marquès, 1984; Lom & Dyková, 1992b).

This was the first Triactinomyxon recorded from the marine environment. At least 25 oligochaetes harboured the parasite. Other hosts than *Limnodriloides* cf. *victoriensis* were ?*Thalassodrilides* sp. (Limnodrilinae) and *Duridrilus* sp. (Phallodrilinae). Infections were difficult to detect without squashing and killing the hosts. Clusters of 3 polar capsules in the intestinal epithelium or a distended intestine are indicative of an infection (Fig. 6B, D). Another 12 oligochaetes had developing Triactinomyxon-like stages in the intestine but no free spores were seen (QM G463607 #203).

### **Triactinomyxon type 1** nov. (Figs 5, 6)

HOST. Immature limnodriloidine oligochaete.

SITE IN HOST. Posterior gut distended with pansporocysts.

LOCALITY. Boggy Creek, 27°24'S, 153°09'E.

DESCRIPTION. Ovoid spores in pansporocyst become anchor-shaped when exposed to

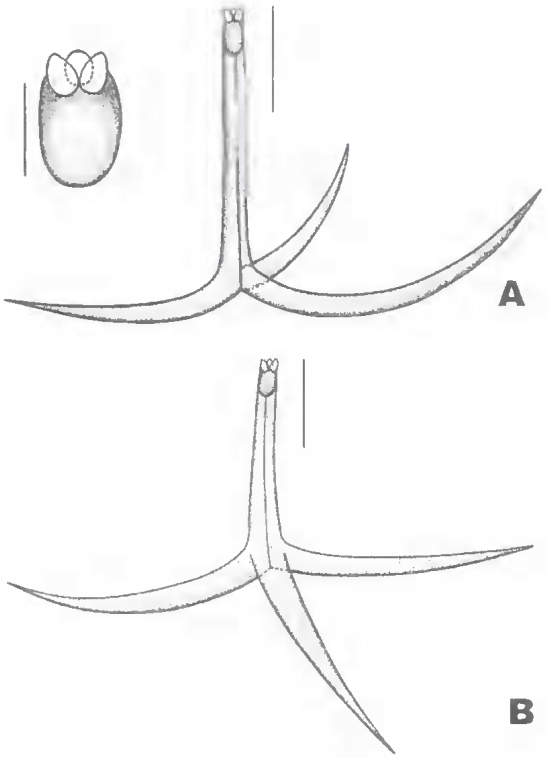



FIG. 5. Drawing of Triactinomyxon types. Side view of spores fully expanded in seawater. A, Triactinomyxon of Roubal et al., 1997. Scale = 50μm. [Inset: Spore in intestinal lumen before contact with seawater and valve expansion. Scale = 10μm.] B, Triactinomyxon type 1 nov. Scale = 100μm.

seawater (Fig. 5B). Polar capsules (8-11μm long) remain at anterior end of spore; valve cells form elongated stylus and 3 caudal processes. Spore body (polar capsules plus sporoplasm) 32-44μm long (34μm, n=20) (Fig. 6C) and total spore length 208-268μm (236μm, n=20). Caudal processes, 240-360μm long (296μm, n=46), curve slightly anteriorly, taper to point, α 90°. Suture lines visible, germ cells indiscernible.

REMARKS. Triactinomyxon type 1 closely resembles Triactinomyxon of Roubal et al., 1997, except it is twice the size. When compared to other types (Marquès, 1984 [8 types]; McGeorge et al., 1997 [1 type]; Xiao & Desser, 1998a [6 types]; El-Mansy et al., 1998b, c [9 types]), including those involved in a myxosporean life cycle (El-Matbouli & Hoffmann, 1989, 1993, 1998; Kent et al., 1993; El-Mansy & Molnár, 1997a, b; El-Mansy et al., 1998a; Székely et al., 1999; Eszterbauer et al., 2000), Triactinomyxon type 1 most closely resembles Triactinomyxon type 4 of El-Mansy et al., 1998c and Triactinomyxon 'E' of Xiao & Desser, 1998a. However, although the length of the processes and polar capsules are similar in type 4 of El-Mansy et al. and our type 1, the average length of the spore body (45μm) is greater and the length of the style (149μm) less for the former than that of the latter. Similarly, the process length of Triactinomyxon 'E' is within the range of Triactinomyxon type 1, but the polar capsules of the former are smaller (5μm) as is the total spore length (spore axis 190–210μm).

**Triactinomyxon type 2** nov.
(Fig. 7)

HOST. Unidentified tubificid oligochaete.

SITE IN HOST. Not possible to determine if infection is coelomic or intestinal; intestine distended with pansporocyst within its boundary, but the latter may be above rather than within intestine.

TYPE LOCALITY. Boggy Creek, 27°24'S, 153°09'E.

DESCRIPTION. Pansporocyst ~139μm across, with 8 subspherical spores (Fig. 7A). Spores fill out into characteristic triactinomyxon anchor-shape after contact with seawater (Fig. 7C). Polar capsules 3, pyriform, ~8 × 6μm, at anterior end of stylus (Fig. 7B). Spore ~346μm long, ~38μm wide, narrowed anteriorly, posterior end dividing into 3 caudal processes directed posteriorly which taper at ends, ~517μm long, α 130°. Sporoplasm within stylus, ~130μm long.

REMARKS. The thick stylus and arms are reminiscent of *Siedleckiella* Janiszewska, 1955 but spores of Triactinomyxon type 2 did not appear interconnected either in the pansporocyst or in seawater and arms of *Siedleckiella* are blunt rather than pointed. Triactinomyxon type 2 is larger than any known Triactinomyxon. The arms of *T. magnum* Granata, 1923 are >500μm but the stylus is only 25-30μm long. The size of the sporoplasm suggests it contains numerous germ cells. Development is asynchronous. Spores were liberated from pansporocysts under pressure. This form was observed on only one occasion and the host worm disintegrated during observation under the cover slip.

Phylum APICOMPLEXA Levine, 1970
Order EUGREGARINORIDA Léger, 1900

Merogony absent; gametogony and sporogony present; typically parasites of annelids and arthropods, but some in other invertebrates.

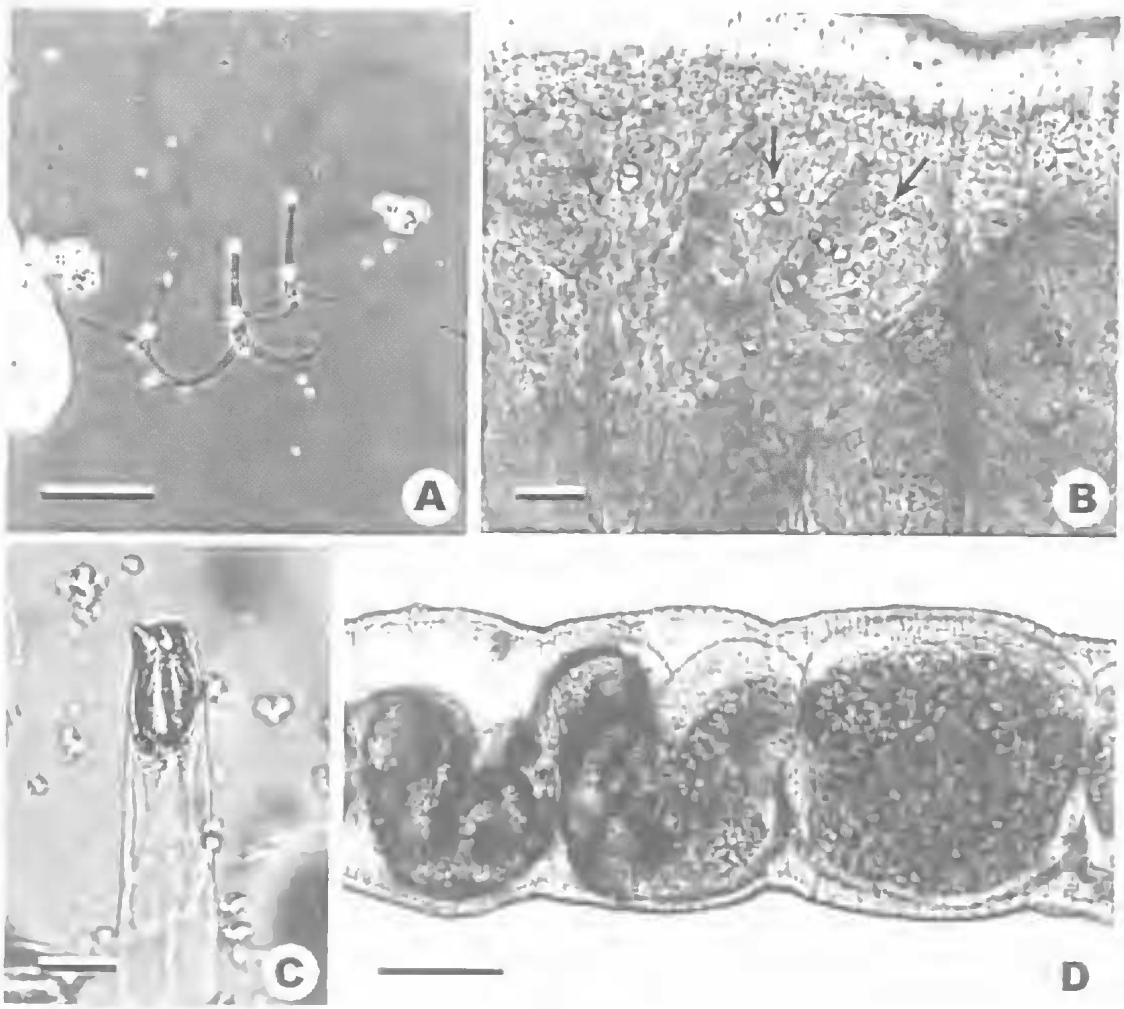



FIG. 6. Triactinomyxon spores. Fresh unstained material. A, Triactinomyxon of Roubal et al., 1997 spores in sea water. Scale = 125µm. B, prominent polar capsules of Triactinomyxon of Roubal et al., 1997 spores within pansporocysts developing in the intestine of an oligochaete. Scale = 20µm. C, anterior end of Triactinomyxon type 1 nov. spore in seawater showing polar capsules and sporoplasm. Scale = 20µm. D, intestine of limnodriloidine oligochaete distended with developing stages of Triactinomyxon of Roubal et al., 1997. Left side uninfected, right side is filled with pansporoblasts. Scale = 200µm.

Family MONOCYSTIDAE Bütschli, 1882

Gamonts spherical to cylindrical, with anterior end little differentiated if at all; oocysts biconical or navicular; mostly coelomic; the great majority are parasites of oligochaetes.

Genus **Oligochaetocystis** Meier, 1956

Gamonts club-shaped, solitary or in syzygy; syzygy head-to-head [type-species: *O. pachydrili* (Lankester, 1863) Meier, 1956 emend. Levine, 1977].

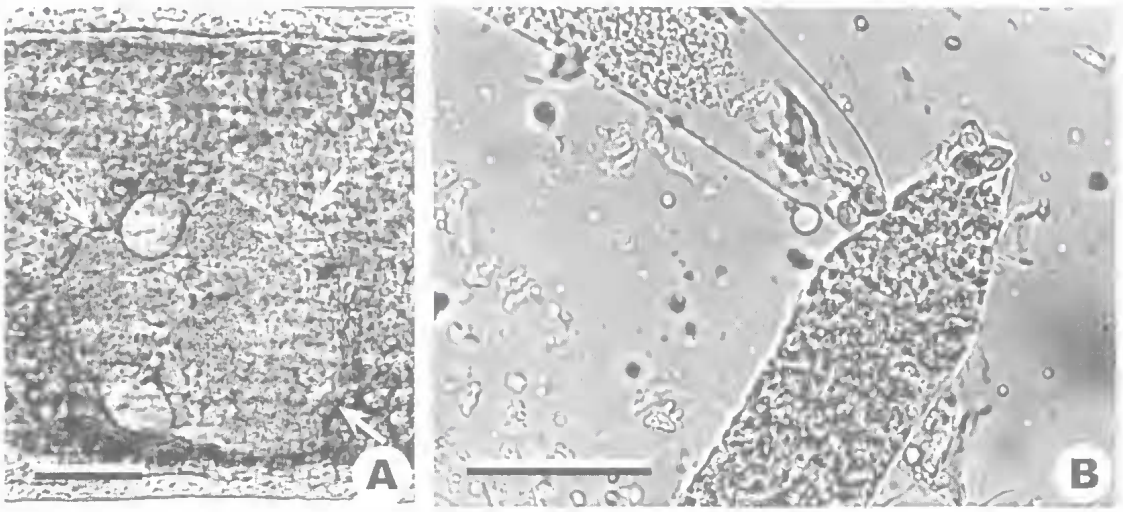
**Oligochaetocystis sp.**
(Fig. 8)

HOST. Immature tubificid oligochaete.

SITE IN HOST. All stages coelomic. Several gamonts appeared attached to the oligochaete intestine by their anterior ends. Infection extended from anterior region to middle of oligochaete.

LOCALITY. Boggy Creek, 27°24'S, 153°09'E.

SPECIMEN LODGED. QM G462725 (#147).



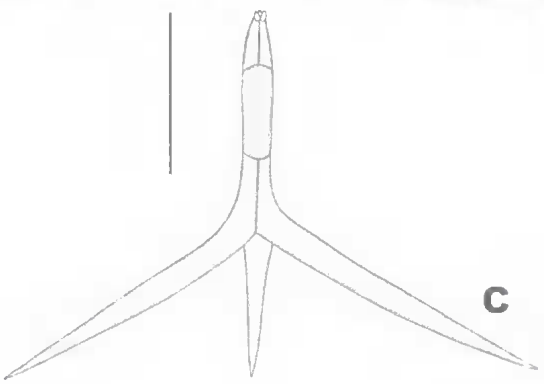




FIG. 7. Triactinomyxon type 2 nov. Fresh unstained material. A, Pansporocyst (bound by arrows) in intestine of a limnodriloidine oligochaete. Scale = 50µm. B, polar capsules and partial sporoplasm of a spore in seawater. Scale = 40µm. C, drawing of side view. Scale = 250µm.

DESCRIPTION. Gamonts (trophozoites) club-shaped and aseptate 148-185 (167) × 31-40 (35)µm (Table 3). Posterior region paddle-like, narrows into a 'neck' that forms a bulb-like anterior region (Fig. 8A,B). Mucron inconspicuous. Nucleus clear, oval to circular, diameter 6-12 (10)µm, in mid-posterior region. Gamonts solitary, granular, syzygy not observed. Gametocysts round (diameter 77-96µm), containing numerous (100+) gametes (Fig. 8C, D).

REMARKS. This is the only record of *Oligochaetocystis* sp. among the oligochaetes examined. The worm also harboured a coelomic actinosporean, *Sphaeractinomyxon ersei*. *Oligochaetocystis* contains 3 species: *mesenchytraei, pachydrili* and *saenuridis* in the coelom of European freshwater oligochaetes; *Mesenchytraeus flavidus*, *Lumbricillus* spp. (Enchytracidae) and *Tubifex tubifex* (Tubificidae) respectively (Levine, 1977,1988). *O. saenuridis* also occurs in the seminal vesicles.

Phylum CILIOPHORA Dolflein, 1901

Internal ciliates were most prevalent in worms from Heron Is.; infected worms were rare in Moreton Bay and were represented usually by a single ciliate. In contrast, ectocommensal ciliates, represented by *Scyphidia* sp., were found only on Moreton Bay oligochaetes.

Class OLIGOHYMENOPHORA de Puytorac et al., 1974

Order ASTOMATIDA Schewiakoff, 1896

Large body; uniformly ciliated; mouthless; endosymbiotic in oligochaetes, polychaetes, leeches, free-living flatworms and molluscs.

Family RADIOPHRYIDAE de Puytorac, 1972

Body flattened; V-shaped apical cytoskeletal organelle; dense somatic ciliation.

**Radiophrya** sp.
(Fig. 9A-C)

HOST. Tubificid oligochaetes.

SITE IN HOST. Intestinal lumen, near clitellar region (Fig. 9A).

SPECIMENS LODGED. QM G463608 (H116), G463609 (H133).

LOCALITY. Heron Is., 23°27'S,151°55'E.

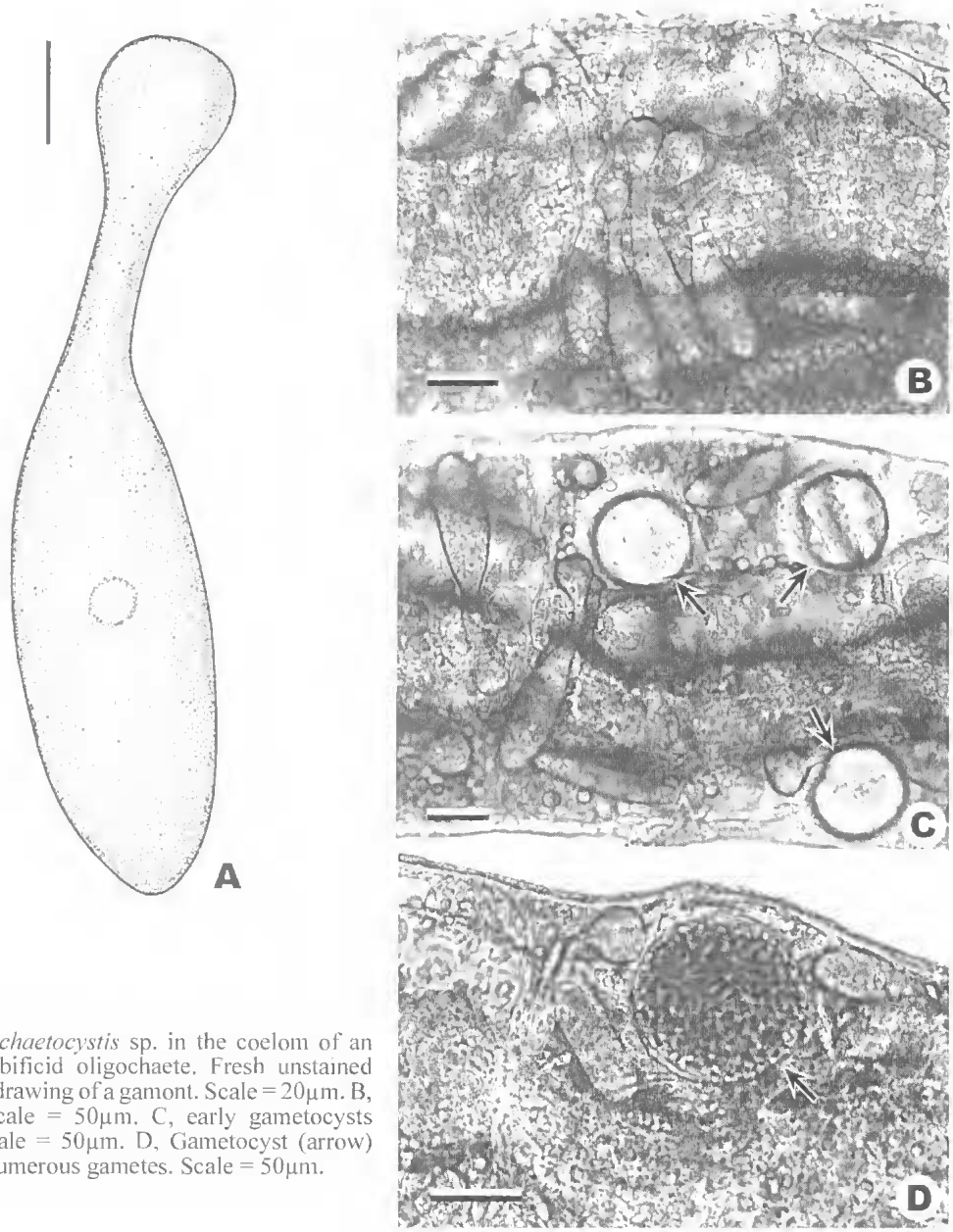



FIG. 8. *Oligochaetocystis* sp. in the coelom of an immature tubificid oligochaete. Fresh unstained material. A, drawing of a gamont. Scale = 20µm. B, Gamonts. Scale = 50µm. C, early gametocysts (arrows). Scale = 50µm. D, Gametocyst (arrow) containing numerous gametes. Scale = 50µm.

DESCRIPTION. Body elongate, straight to curved, 140-180µm long, tapering posteriorly and anteriorly, V-shaped attachment structure anteriorly, arms of V of about equal length, longitudinal kineties converge at each end; macronucleus large; much of one surface reinforced by cortical fibres extending almost whole length of body (Fig. 9B, C).

REMARKS. Endocommensal, up to 30 per host. Cilia beating.

**Unidentified astomate ciliates**
(Fig. 9D-F)

HOST. *Grania* sp. (Enchytraeidae)

LOCALITY. Heron Is., 23°27'S, 151°55'E.

SITE IN HOST. Intestinal lumen (Fig. 9D).

SPECIMEN LODGED. QMG463610 (H153).

DESCRIPTION. Ciliates elongate, 177-257µm long, 65µm wide, posteriorly tapered, anteriorly blunt, oral structures (vestibulum, cytostome, trichites) absent, no prominent anterior attachment region or structures such as suckers, hooks or spines; macronucleus elongate ribbon-like; kineties longitudinal, ~10 rows (Fig. 9E, F). Budding not observed.

REMARKS. All characters conform to those of the Astomatida but none conforms exactly to any of the 8 families of astomate ciliates known from aquatic and terrestrial annelids. Apparent lack of an anterior attachment structure is consistent with the Anoplophryidae but most species in this family are large (>300µm) and have about 40-100 kineties, whereas the present ciliates are up to 260µm long and have only 10 longitudinal kineties. Species belonging to all other families have distinctive attachment organelles. At least 6 ciliates inhabited the worm with only 1 per segment.

TABLE 3. Morphometric characters of the gregarine *Oligochaetocystis* sp.

| | Mean (µm) | Minimum (µm) | Maximum (µm) | No. observations |
|---|---|---|---|---|
| GAMONT | | | | |
| Length | 166.9 | 148.3 | 185.4 | 8 |
| Width | 34.8 | 30.9 | 40.2 | 8 |
| Neck width | 10.3 | 7.7 | 13.9 | 8 |
| Bulb width | 24.7 | 24.7 | 24.7 | 3 |
| Bulb length | 25.7 | 21.6 | 27.8 | 3 |
| Nucleus diameter | 10.3 | 6.2 | 12.4 | 3 |
| GAMETOCYST | | | | |
| Early | 81.4 | 77.3 | 86.5 | 3 |
| Late | 95.8 | - | - | 1 |

Order PERITRICHIDA Stein, 1859

Body goblet-shaped; conspicuous oral ciliature winding counter-clockwise to cytostome; scopula antapical; widespread throughout aquatic habitats; many free-living or symphorionts on diverse hosts; some commensals or parasites.

Family SCYPHIDIIDAE Kahl, 1933

Solitary zooids; stalkless; disc-like scopula; generally found as epibionts mainly on invertebrates.

**Scyphidia** sp.
(Fig. 10)

HOST. Limnodriloidine oligochaetes.

SITE IN HOST. Attached to posterior integument (Fig. 10A).

LOCALITY. Boggy Creek, Moreton Bay, 27°24'S, 153°09'E.

DESCRIPTION. Zooid bell-shaped, ~40-50µm long, aloricate, stalk absent; scopula sessile, solitary (but can occur in close proximity to each other), macronucleus U-shaped (Fig. 10A).

REMARKS. This ectocommensal ciliate was found attached to the posterior region of oligochaetes from muddy habitats but was never associated with oligochaetes from the coral reef. About 10 ciliates were present simultaneously on a worm either singly or in small groups of 2-5 zooids. *Scyphidia* spp. attach to both vertebrates and invertebrates as well as to submerged objects; about 19 species have been recorded.

Phylum HAPLOSPORIDIA Caullery & Mesnil, 1889

Histozoic, coelozoic unicellular parasites which form unicellular, typically uninucleated distinctive propagules, 'spores' without polar capsules or polar filaments (Perkins, 1990).

**Haplosporidium** sp.
(Fig. 10)

HOST. Tubificid oligochaetes (including *Heterodrilus* sp.).

SITE IN HOST. Intestinal epithelium, free spores in intestinal lumen.

LOCALITY. Heron Is., 23° 27'S, 151° 55'E.

SPECIMENS LODGED. QM G463611 (H97), QM G463612 (H147).

DESCRIPTION. Unicellular spore without polar capsule or polar filament. Spores irregularly shaped, round to oval, 8-10 × 13-15µm, groups of about eight enclosed by membrane (pansporoblast ~23µm) (Fig. 10B). Spores possess characteristic operculum-like caps.

REMARKS. Haplosporidia were first believed to be actinosporeans because of the arrangement of the spores in groups of eight in the intestine; however, they do not contain polar capsules. Sixteen of the 2,000 oligochaetes examined from Heron Is. harboured *Haplosporidium* sp.

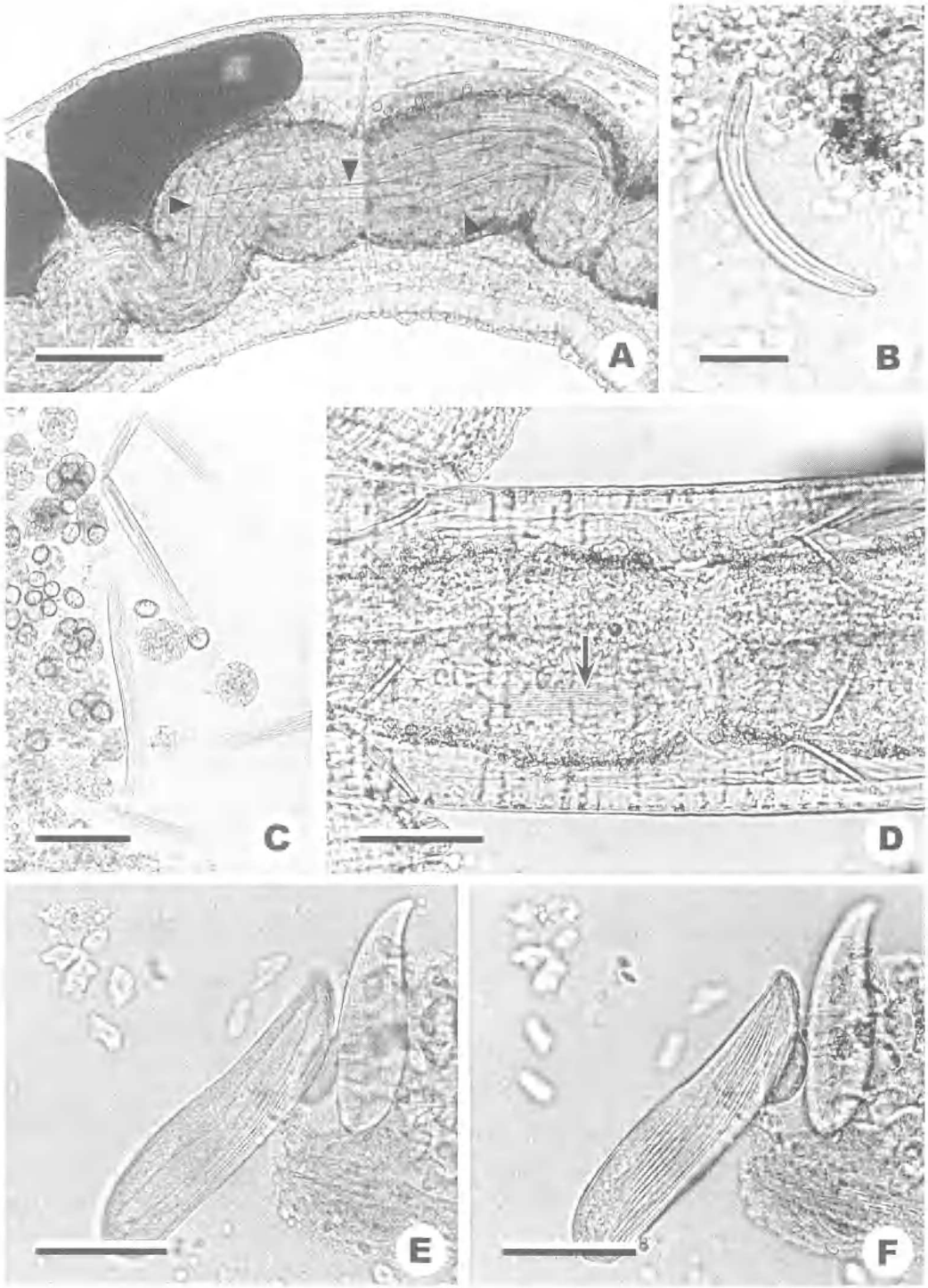



FIG. 9. Astomate ciliates. A-C, *Radiophyra* sp. D-F, unidentified, from the intestinal lumen of oligochaetes at Heron Island. Fresh unstained material. A, ciliates in intestinal lumen of a tubificid oligochaete (arrowheads). Scale = 150μm; B, curved ciliate in seawater. Scale = 50μm; C, elongate ciliates in sea water. Scale = 100μm; D, ciliate (arrow) in the intestinal lumen of an enchytraeid oligochaete. Scale = 100μm; E, ciliate in seawater with macronuclei in focus. Scale = 100μm; F, same ciliates with somatic kineties in focus. Scale = 100μm.

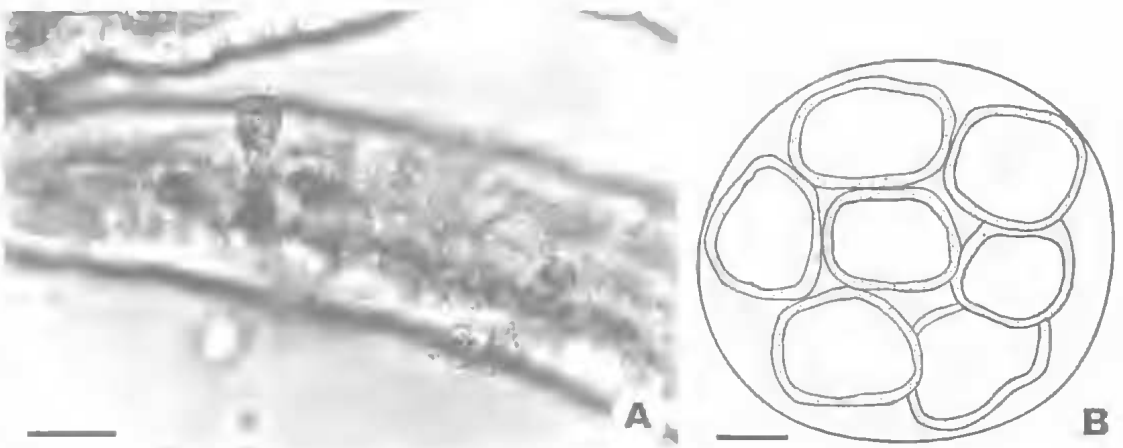



FIG. 10. Protozoans from marine oligochaetes. A, *Scyphidia* sp. contracted zooids attached to posterior region of limnodriloidine oligochaete. Fresh unstained material. Scale = 50µm; B, *Haplosporidium* sp. pansporoblast containing eight spores. Scale = 5µm.

Phylum APICOMPLEXA Levine, 1970
Class COCCIDEA Leuckart, 1879

Life cycle with merogony, gamogony and sporogony; producing small intracellular gamonts, single macrogamete; monoxenous or heteroxenous parasites in vertebrates and invertebrates.

**Unidentified coccidian**
(Fig. 11)

HOST. Tubificid oligochaetes including Limnodriloidinae sp.

SITE IN HOST. Primarily coelomic but the parasites appear to 'bud off' from intestine (Fig. 11A).

LOCALITY. Moreton Bay, 27°15-25'S

DESCRIPTION. Oocysts not seen or not present, sporocysts spherical, 12-16µm (14µm, n=8) in diameter; contain ~16 sporozoites, roughly spherical, ~3µm across (Fig. 11A-C). Meronts and gamonts not observed.

REMARKS. An accurate record of prevalence was not maintained. Parasites were recorded also from the posterior part of the gut. Some sporocysts were observed to 'excyst' in seawater (Fig. 11D). The cysts resemble those of the protococcidian *Grellia* Levine, 1973 which has ellipsoidal sporocysts 12-14µm long, contain 5-14 sporozoites and which inhabits the coelom of archiannelids and polychaetes. The specimens we found had neither ellipsoidal oocysts nor large gamonts. The sporocysts may represent a hitherto unidentified eucoccidian genus.

Phylum NEMATODA
Class ENOPLEA
Order MERMITHIDA
Family MERMITHIDAE

Adult worms free-living; juveniles parasitic in body cavity of various invertebrates, primarily insects; no functional gut at any stage.

**Unidentified mermithid nematodes**
(Fig. 12)

HOST. *Heterodrilus* cf. *keenani* and possibly other tubificid taxa.

SITE IN HOST. Coelom, in posterior part of host.

LOCALITY. Heron Is., 23° 27'S, 151° 55'E.

SPECIMEN LODGED. QM G218270 (H145).

DESCRIPTION. Two types of mermithid nematodes were observed; both juvenile (no gonads) and coiled in the host (Fig. 12). Width uniform along length. Anterior end blunt, posterior end tapered to a point. Buccal cavity, short, narrow, anterior end rounded, reminiscent of ascarophid nematodes. Pharynx, not well defined; lips absent. One nematode type long with green intestine, four specimens, length 1,400-2,064µm, width 30-41µm (Fig. 12A). Second type short, without pigmentation, two specimens, length 244-1,080µm, width 10-35µm (Fig. 12B).

REMARKS. Four oligochaetes each harboured 1 nematode and a fifth oligochaete held 2 nematodes, one of each type. The nematodes were fixed inside the oligochaete host. Some

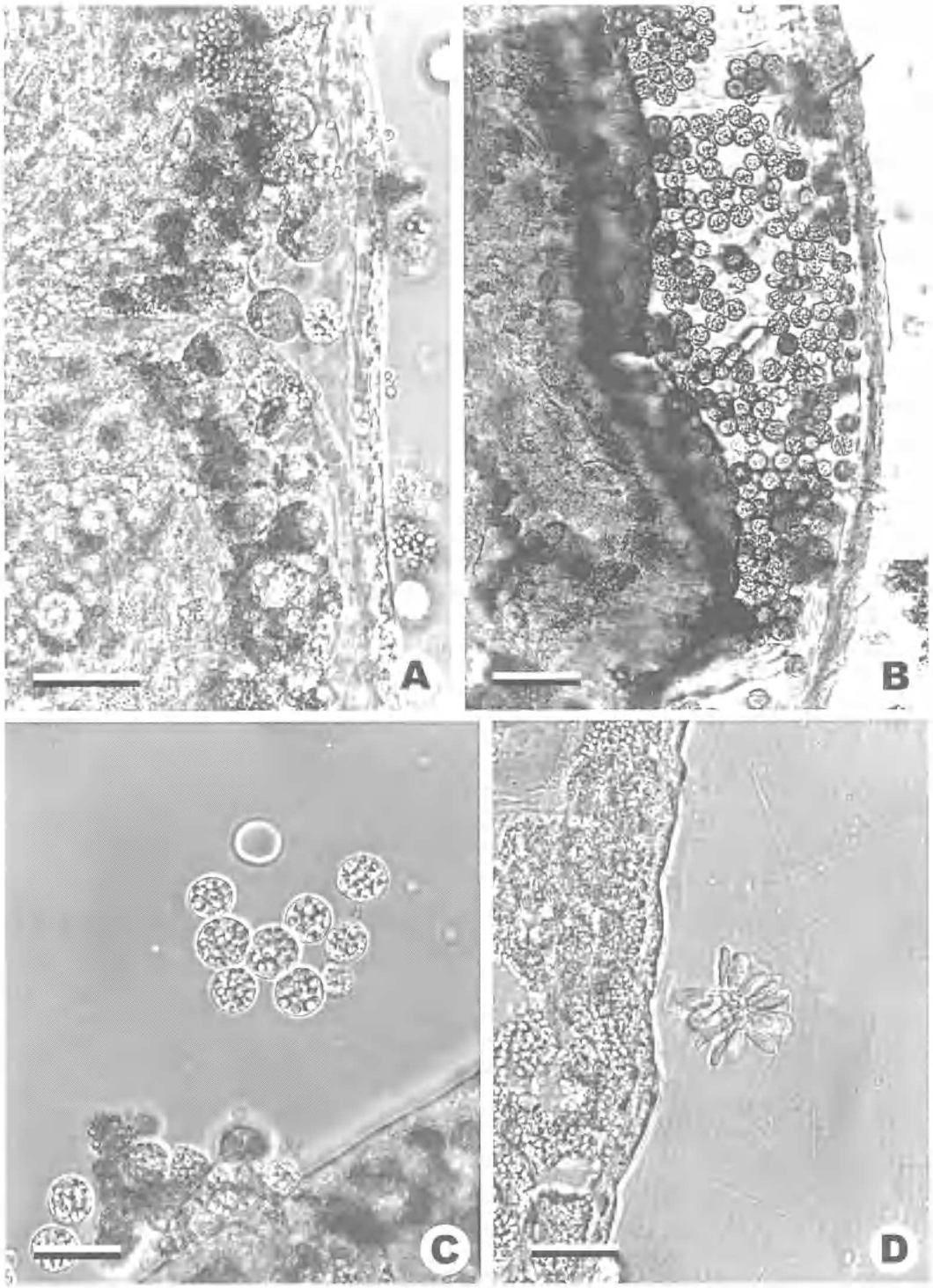



FIG. 11. Unidentified coccidian from limnodriloidine oligochaete. Fresh unstained material. A, coccidian sporoblasts 'budding-off' intestinal epithelium into coelom. Scale = 30µm; B, numerous sporocysts in coelom. Scale = 50µm; C, sporocysts in seawater. Scale = 25µm; D, excysted sporocyst with sporozoites netted in seawater. Scale = 25µm.

were then cleared and stained with chlorlactophenol and Mayer's haemotoxylin but this did not facilitate identification of them.

## DISCUSSION

Australian marine oligochaetes were found to be infected with a range of metazoan and protozoan parasites/commensals (Tables 1, 2). Tubificids dominated the oligochaete samples and although enchytraeids comprised at least 11% of the Heron Is. collections, only one worm was infected (*Grania* sp. with an astomate ciliate). Members of the tubificid Limnodriloidinae, Phallodrilinae and Rhyacodrilinae were infected with actinosporeans, ciliates, haplosporidians, coccidians, nematodes and gregarines. While double infections with species from the same parasite group were rare, infections by two parasites belonging to different groups were not uncommon; e.g. actinosporeans and ciliates or actinosporeans and gregarines.

MYXOZOA. The actinosporeans we described (except possibly Triactinomyxon of Roubal et al., 1997) have been found only in Australia. Marine actinosporeans do not exhibit the same degree of host specificity as do freshwater actinosporeans and most (>60%) infect 2 or more marine oligochaetes e.g. *Sphaeractinomyxon ersei* was recorded from *Doliodrilus*, *Limnodriloides*, *Thalassodrilides* and *Bathydrilus*. Conversely, a species of marine oligochaete may be host to several actinosporean types. e.g. *Thalassodrilides* is infected by both *S. ersei* and *Endocapsa rosulata* but, as far as we have observed, not simultaneously. In contrast, 90% of freshwater actinosporeans occur in only one oligochaete species (cf. Marquès, 1984; Xiao & Desser, 1998c). One oligochaete species, however, may be host to several actinosporeans e.g. *Tubifex tubifex* is host to at least 12 types represented by 7 collective groups: Neoactinomyxum, Guyenotia, Echinactinomyxon, Raabeia, Triactinomyxon, Hexactinomyxon and Synactinomyxon.

The role of actinosporeans in marine oligochaetes is not yet fully understood but both are implicated in the life cycle of marine myxosporeans. Of particular importance are members of the myxosporean genus *Kudoa* that dwell in the skeletal muscles of marine fish and cause economic losses in mariculture around the world (Ireland, USA, Canada and Australia) (see Kent et al., 1994b; Palmer, 1995; Hallett et al., 1997b). Knowledge of the biology of the parasite, including its life cycle, is required to control and alleviate these problems. Despite evidence that at least 24 freshwater myxosporeans alternate with an actinosporean stage in an oligochaete, similar connections (or any others) have not been established for any marine myxosporeans or actinosporeans. Indeed, Diamant (1997) provides experimental evidence for direct fish-to-fish transmission of at least 1 species, *Myxidium leei*, which would theoretically eliminate the need for an alternate invertebrate host (Diamant, 1997); though this direct transmission may alternate with transmission via actinospores (Lom & Dyková, 1995).

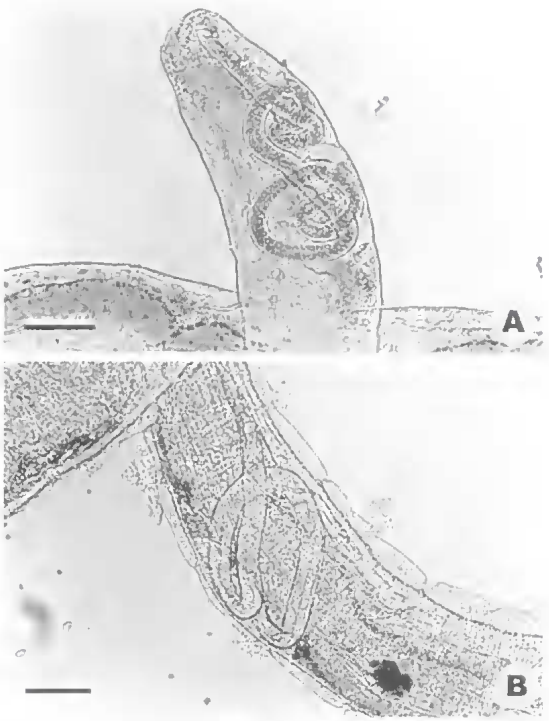



FIG. 12. Mermithid nematodes in posterior coelom of oligochaetes from Heron Island. Fresh unstained material. A, green mermithid nematode. Scale = 100μm. B, clear mermithid nematode. Scale = 100μm.

Significant changes were proposed for the taxonomic treatment of actinosporeans during the course of our studies. The result of conclusions made by Kent et al. (1994a) that alternate myxosporean development probably occurs in all actinosporean families and genera, was the proposal that nominal actinosporean generic names should not be distinguished from

myxosporean genera and, consequently, all actinosporean genera and species be declared invalid (except *Tetractinomyxon*) thereby treating their nominal generic names as collective group names. The majority of actinosporean descriptions postdating these proposals adopt them without comment (McGeorge et al, 1997; Xiao & Desser, 1998; El-Mansy et al., 1998b,c). Various researchers over the past two decades have proposed the redundancy, either partly or fully, of every taxonomic level within the Myxozoa, notwithstanding the phylum itself (Kent et al., 1994a; Siddall et al., 1996). The Myxozoa are now recognised generally as being Metazoa, although their exact placement within this group is still unclear. Because the position of most actinosporeans within the phylum is currently uncertain, and for the sake of consistency, we employ the identification system recommended by Kent et al. (1994a) and Lom et al. (1997), although we would prefer to ascribe a binomial identity to actinosporean forms that do not have clear links to myxosporean genera. We have added 'nov.' at the start of descriptions of previously undescribed forms to avoid confusion between new forms and those already described (cf. El-Mansy et al., 1998c).

Prior to this study, marine actinosporeans had been described from France (*S. stolci* from the oligochaete *Clitellio arenarius* and *Henitubifex benedeni* (= *Tubificoides benedii*) (Caullery & Mesnil, 1904; Marquès, 1984)), Romania (*S. stolci* from *Tubifex* sp. (Radulescu & Motilicia, 1957)), England (*Tetractinomyxon intermedium* and *T. irregulare* from the sipuneulid worm *Petalostoma minutum* (Ikeda, 1912)) and Hong Kong (*Aurantiactinomyxon* type 1 & 2 from *Pacifidrilus vanus*, *Aurantiactinomyxon* type 2 from *P. darvelli* and *Limnodriloides toloensis*, *Sphaeractinomyxon* type 1 from *Aktedrilus mortoni* and *Sphaeractinomyxon* type 2 from *Ainudrilus geminus* (Hallett et al., 1997a)). We have now observed marine actinosporeans not only in Australian tubificid oligochaetes but also marine oligochaetes from: near Honiara, Solomon Islands (Tubificidae sp. (unidentified), Limnodriloidinae sp. and *Heterodrilus* sp.); Jiaozhou Bay, near Qingdao, China (*Doliodrilus tener*); Florida, USA (*Tectidrilus squalidus*); and Ascension Island, South Atlantic (*Thalassodrilides gurwitschi*) (all material retrieved from second author's collection; unpubl. data).

This study demonstrates that actinosporeans occur in marine oligochaetes, and these findings will facilitate experimental and molecular studies into the life cycle and systematics of this group.

Infections in oligochaetes could not be detected on the basis of differences in worm motility, colour, size or shape. Instead, coelomic infections were readily discerned by the presence of iridescent spheres in the coelom when examined using incident light under a dissection microscope (the refractile bodies representing pansporocystic stages of the actinosporeans). The sporogonic stages varied considerably in size and internal composition and were more difficult to recognise, but the presence of numerous mature spores, monomorphic in appearance, was indicative of infection. Light microscopy generally revealed the coelom to be packed with pansporocysts in coelomic infections or the intestine to be distended with pansporocysts in gut infections. Similar distension has been reported also by Janiszewska (1955). Wolf et al. (1986), however, found the intestinal (freshwater) actinosporean *Triactinomyxon gyrosalmo* to be abundant in worms that were pale, had generalised anterior swellings and displayed an opaque outer layer. Similarly, Molnár et al. (1999a) could distinguish tubificids heavily infected with raabeia to be pale in colour and move sluggishly. El-Matbouli & Hoffmann (1993) recognised triaetinomyxon infected tubificids by their whitish discolouration and Molnár et al. (1999b) noted that intestinal segments infected with neoactinospores appeared darker in colour and had thickened walls compared to uninfected areas. In contrast, Yokoyama et al. (1991) found that *Raabeia* sp. infections, apparently in the body cavity of the freshwater tubificid *Branchiura sowerbyi*, were not visible externally. We found coelomic infections to develop anteriorly in an oligochaete with more advanced stages located more posteriorly as the infection developed. El-Mansy et al. (1998a) observed triactinospores in the centrally located intestinal segments in moderate infections but in most segments when severe.

The findings presented in this review support the literature that natural actinosporean infections have a low prevalence in oligochaetes being 0.1-9.5% (Mackinnon & Adam, 1924; Hamilton & Canning, 1987; Yokoyama et al., 1991, 1993a, 1993b; McGeorge et al., 1997; Hallett et al., 1998; Xiao & Desser, 1998c; Özer & Wootten, 2000). An exception is the findings of El-Mansy et al. (1998c) who recorded a significantly higher prevalence of up to 43% which they attributed to their examination

technique. Mixed infections are reported to be rare (Yokoyama et al., 1991; Xiao & Desser, 1998c) and indeed, only one of the 222 infected worms of the present study harboured two actinosporean species. In experimental infections, prevalences of 3 to almost 100 percent have been observed (Wolf et al., 1986; Kent et al., 1993; Yokoyama et al., 1993a; Uspenskaya, 1995; El-Mansy et al., 1997b; El-Mansy et al. 1998a; Molnár et al. 1999a,b; Özer & Wootten, 2000). Coelomic actinosporeans accounted for most infections (>80%) and *Sphaeractinomyxon ersei* represented 23.9% of all infected worms from Moreton Bay. In contrast, all 25 actinosporeans recorded by Xiao & Desser (1998c) parasitised the intestinal epithelium. Gut-inhabiting actinosporeans like *Neoactinomyxum*, *Guyenotia* and *Hexactinomyxon* spp., are common in freshwater oligochaetes, however the only gut-inhabiting actinosporeans we identified were forms of Triactinomyxon. There was no obvious seasonal influence on infections; the highest prevalence (12.1%) was recorded in June at Boggy Creek and the lowest (0.47%) in May at Scott Point. A number of other studies, however, have reported temporal patterns (see Yokoyama et al., 1993; Xiao & Desser, 1998c; El-Mansy et al., 1998c; Özer & Wootten, 2000).

Most infected oligochaetes were sexually immature, which hindered identification of the host. It is not known whether the presence of actinosporeans and other parasites may adversely affect the maturation of oligochaetes. Sexually mature worms, however, constitute only a small part of populations of marine oligochaetes at most times (Erséus, 1994).

Different fixatives (including ethylalcohol, Bouin's, Trump's, glutaraldehyde and Karnovsky's) were used to preserve infected worms, depending upon their intended use. These chemicals had varying effects on actinosporean spores when combined with the stain paracarmine and the mounting process which were necessary to identify the host worms. Karnovsky's fixative preserved spores best even though they had 'shrunk' by 1-2µm (3.5%); all other fixatives resulted in greater shrinkage of the spores. *Endocapsa* specimens were affected to a greater extent than *Sphaeractinomyxon* or *Tetraspora* types, related perhaps to the valve cell properties of these groups; *Endocapsa* species have valve cells that swell whereas those of *Sphaeractinomyxon* and *Tetraspora* types do not (see Hallett et al., 1998; Hallett et al., 1999; Hallett & Lester, 1999). Detailed drawings accompanied with a range of micrographs are therefore recommended (see also Lom et al., 1997). Material fixed in Bouin's or Trump's fixative, but not processed further, appear to be representative but spores become deformed and distorted when stained and mounted. A complete taxonomic description requires information about: mature spores observed both in the host and free in seawater (or freshwater) to monitor changes in size and shape; the host stained in alcoholic paraearmine and mounted whole in Canada Balsam preferably after fixation in Bouin's solution; and developing stages and where they are located in the host and their appearance. The anterior part of the oligochaete fixed separately in Bouin's solution is needed to check host identity regardless of the intended use of the infected worm for either histology, TEM or DNA studies.

APICOMPLEXA. Gregarines are widespread, common parasites of invertebrates, particularly arthropods. New hosts ingest gametocysts and the oocysts they contain, to become infected. The low prevalence of gregarines in marine oligochaetes suggests they may be an atypical host group; nevertheless gregarines formed gametocysts and developed. Apparently, oocysts were ingested and sporozoites migrated across the gut epithelium into the body cavity of the host worm where they underwent gamogony, but neither oocysts containing sporozoites nor syzygy were observed. We believe this to be the first record of a marine tubificid oligochaete infected with a gregarine, but enchytraeids infected by monocystid gregarines dominate the records (Giere & Pfannkuche, 1982).

CILIOPHORA. In this study, most ciliates were fixed *in situ* within worms so that the hosts could be identified. Subsequent dissections of infected worms yielded few intact ciliates and silver impregnation studies were uninformative. None of the ciliates were therefore identified to species level. Nonetheless, the ciliates were clearly astomate and peritrichous species as determined by their morphological characters. Similar groups occur as endo- or ecto- commensals in oligochaetes (Giere & Pfannkuche, 1982). Ciliates should be carefully extracted from host tissues and observed live to note colour, rigidity, motility, contractile vacuole, location, etc. Ciliates should then be fixed in Bouin's, Stieve's or Dafano's fluid prior to silver impregnation to reveal patterns of ciliation, attachment structures, nuclear arrangement, etc. Regrettably, the best

silver stain to use for any particular ciliate group can vary considerably so it is advisable to use multiple stains including silver nitrate, silver carbonate and silver proteinate. The prevalences and intensities of infection by internal ciliates were lower in Moreton Bay than at Heron Island. External ciliates, however, were observed only on worms from Moreton Bay, particularly those collected at Boggy Creek with substantial silt loads compared to the pristine coral cay of Heron Island; the parasite fauna reflects this difference. Similar external ciliates (order Sessilida) were reported from *Limnodriloides biforis* Erséus, 1990 in muddy sediments associated with estuarine habitats in Hong Kong (Erséus, 1990). *Grania* spp. (Enchytraeidae) for some reason seem untouched by the parasites which were relatively prevalent in marine tubificids.

NEMATODA. Oligochaetes are phoretic, paratenic, intermediate and definitive hosts for nematodes (Poinar, 1978; Smith, 1985). Juvenile mermithid nematodes primarily infect insects but also molluscs, crustaceans, arachnids and other invertebrates; mermithid adults are free-living (Poinar, 1976). The juvenile emerges from the egg, penetrates into the body cavity of an invertebrate host, develops for a period to emerge finally into the environment where it moults to the adult stage. Mermithids almost always kill their host (Poinar, 1976); the large size of the nematodes relative to their oligochaete host observed in the current study imply a similar life cycle for the mermithids we found.

The majority of associations of nematodes and oligochaetes are with earthworms (Poinar, 1978). Only 1 of the 83 species cited in Poinar (1978) was a tubificid (freshwater) oligochaete and its nematode was listed as unidentified. Smith (1985) subsequently documented at least 3 microdrile families as hosts to this group; these were Lumbriculidae, Naididae and Tubificidae to members of Dioctophymatidae, Rhabditidae, Anisakidae and Mermithidae. Smith (1985) found Mermithidae usually in the anterior half of the naidid oligochaetes examined. All six nematodes we found were located posteriorly in the tubificid oligochaetes. Smith (1985) considered migration to be unlikely but rather assumed that the larvae hatched quickly from ingested eggs, penetrated the gut and then resided in the anterior portion of the worm. No more than two nematodes were observed per worm in either study and Smith (1985) recorded that the total prevalence of infection was low and concluded that naidid infections were probably incidental.

Parasitology of marine oligochaetes is fertile ground for studies and we made many new records of infection. The diverse range of organisms detected in oligochaetes indicate that worms are susceptible to external, intestinal and coelomic infections by both commensal and parasitic species. The host range, geographic distribution, habitat requirements and specificity of infection for parasites of marine oligochaetes remain to be determined.

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## LITERATURE CITED

CAULLERY, M. & MESNIL, F. 1904. Sur un type nouveau (*Sphaeractinomyxon stolci* n.g. n.sp.) d'Actinomyxidies, et son développement. Comptes rendus des séances de la Societé de biologie Paris 56: 408-410.

DIAMANT, A. 1997. Fish-to-fish transmission of a marine myxosporean. Diseases of Aquatic Organisms 30: 99-105.

EL-MANSY, A. & MOLNÁR, K. 1997a. Extrapiscine development of *Myxobolus drjagini* Akhmerov, 1954 (Myxosporea: Myxobolidae) in oligochaete alternative hosts. Acta Veterinaria Hungarica 45: 427-438.

1997B. Development of *Myxobolus hungaricus* (Myxosporea: Myxobolidae) in oligochaete alternate hosts. Diseases of Aquatic Organisms 31: 227-232.

EL-MANSY, A., MOLNÁR, K. & SZÉKELY, C.S. 1998a. Development of *Myxobolus portucalensis* Saraiva & Molnár, 1990 (Myxosporea: Myxobolidae) in the oligochaete *Tubifex tubifex* (Müller). Systematic Parasitology 41: 95-103.

EL-MANSY, A., SZÉKELY, C.S., MOLNÁR, K. 1998b. Studies on the occurrence of actinosporean stages of fish myxosporeans in a fish farm of Hungary, with the description of triactinomyxon, raabeia, aurantiactinomyxon and neoactinomyxon types. Acta Vet Hungarica 46:259-284.

1998c. Studies on the occurrence of actinosporean stages of myxosporeans in Lake Balaton, Hungary, with the description of triactinomyxon, raabeia, and aurantiactinomyxon types. Acta Vet Hungarica 46: 437-450.

EL-MATBOULI, M. & HOFFMANN, R.W. 1989. Experimental transmission of two *Myxobolus* spp. developing bisporogeny via tubificid worms. Parasitology Research 75: 461-464.

1993. *Myxobolus carassii* Klokaceva, 1914 also requires an aquatic oligochaete, *Tubifex tubifex* as an intermediate host in its life cycle. Bulletin of the European Association of Fish Pathologists 13: 189-192.

1998. Light and electron microscopic studies on the chronological development of *Myxobolus cerebralis* to the actinosporean stage in *Tubifex tubifex*. International Journal for Parasitology 28: 195-217.

ERSÉUS, C. 1976. Littoral Oligochaeta (Annelida) from Eyjafördur, north coast of Iceland. Zoologica Scripta 5: 5-11.

1990. Marine Oligochaeta of Hong Kong. Pp. 259-335. In Morton, B. (ed.) Proceedings of the Second International Marine Biological Workshop: The Marine Flora and Fauna of Hong Kong and Southern China, Hong Kong, 1986. (Hong Kong University Press: Hong Kong).

1994. The Oligochaeta. Pp. 5-38. In Blake, J.A. & Hilbig, B. (eds) Taxonomic atlas of the Santa Maria Basin and western Santa Barbara Channel. Vol. 4 - The Annelida Part 1. Oligochaeta and Polychaeta: Phyllodocida (Phyllodocidae to Paralacydoniidae). (Santa Barbara Museum of Natural History: Santa Barbara, California).

ESZTERBAUER, E., SZÉKELY, C.S., MOLNÁR, K. & BASKA, F. 2000. Development of *Myxobolus bramae* (Myxosporea: Myxobolidae) in an oligochaete alternate host, *Tubifex tubifex*. Journal of Fish Diseases 23: 19-25.

GIERE, O. & PFANNKUCHE, O. 1982. Biology and ecology of marine Oligochaeta, a review. Oceanography and Marine Biology, an Annual Revue 20: 173-308.

HALLETT, S.L., ERSÉUS, C. & LESTER, R.J.G.1995. An actinosporean from an Australian marine oligochaete. Bulletin of the European Association of Fish Pathologists 15: 168-171.

1997a. Actinosporea from Hong Kong marine Oligochaeta. Pp. 1-7. In Morton, B. (ed.) Proceedings of the Eighth International Marine Biological Workshop: the Marine Flora and Fauna of Hong Kong and Southern China, Hong Kong, 2-20 April, 1995. (Hong Kong University Press: Hong Kong).

1999. Actinosporeans (Myxozoa) from marine oligochaetes of the Great Barrier Reef. Systematic Parasitology 44: 49-57.

HALLETT, S.L. & LESTER, R.J.G. 1999. Actinosporeans (Myxozoa) with four developing spores within a pansporocyst: *Tetraspora discoidea* n. g. n. sp. and *Tetraspora rotundum* n. sp. International Journal for Parasitology 29: 419-427.

HALLETT, S.L., O'DONOGHUE, P.J. & LESTER, R.J.G. 1997b. Infections by *Kudoa ciliatae* (Myxozoa: Myxosporea) in Indo-Pacific whiting *Sillago* spp. Diseases of Aquatic Organsims 30: 11-16.

1998. Structure and development of a marine actinosporean, *Sphaeractinomyxon ersei* n. sp. (Myxozoa). Journal of Eukaryotic Microbiology 45: 142-150.

HAMILTON, A.J. & CANNING, E.U. 1987. Studies on the proposed role of *Tubifex tubifex* (Müller) as an intermediate host in the life cycle of *Myxosoma cerebralis* (Hofer, 1903). Journal of Fish Diseases 10: 145-151.

IKEDA, I. 1912. Studies on some Sporozoan parasites of Sipunculoids. Archiv für Protistenkunde 25: 240-272.

JANISZEWSKA, J. 1955. Actinomyxidia. Morphology, ecology, history of investigations, systematics, development. Acta Parasitologica Polonica 2: 405-437.

1957. Actinomyxidia II. New systematics, sexual cycle, description of new genera and species. Zoologica Polonaie 8: 3-34.

KENT, M.L., MARGOLIS, L. & CORLISS, J.O. 1994a. The demise of a class of protists: taxonomic and nomenclatural revisions proposed for the protist phylum Myxozoa Grassé, 1970. Canadian Journal of Zoology 72: 932-937.

KENT, M.L., MARGOLIS, L., WHITAKER, D.J., HOSKINS, G.E. & MCDONALD, T.E. 1994b. Review of Myxosporea of importance in salmonid fisheries and aquaculture in British Columbia. Folia Parasitologica 41: 27-37.

KENT, M.L., WHITAKER, D.J. & MARGOLIS, L. 1993. Transmission of *Myxobolus arcticus* Pugachev & Khokhlov, 1979, a myxosporean parasite of Pacific salmon, via a triactinomyxon from the aquatic oligochaete *Stylodrilus heringianus* (Lumbriculidae). Canadian Journal of Zoology 71: 1207-1211.

KOSTE, W. 1970. Über eine parasitische Rotatorienart *Albertia reichelti* nov. spec. Zoologischer Anzeiger 184: 428-434.

LEVINE, N.D. 1977. Revision and checklist of the species of the aseptate gregarine family Monocystidae. Folia Parasitologica (Praque) 24 (1).

1988. The protozoan phylum Apicomplexa, 1. (CRC Press Inc.: Florida).

LOM, J. 1990. Phylum Myxozoa. Pp. 36-52. In Margolis, L., Corliss, J.O., Melkonian, M. & Chapman, D.J.(eds) Handbook of protoctista. (Jones & Bartlett: Boston).

LOM, J. & DYKOVÁ, I. 1995. Myxosporea (Phylum Myxozoa). Pp. 97-148. In Woo, P.T.K. (ed.) Fish diseases and disorders Volume 1: Protozoan and Metazoan infections. (CAB International: United Kingdom).

LOM, J., McGEORGE, J., FEIST, S.W., MORRIS, D. & ADAMS, A. 1997. Guidelines for the uniform characterisation of the actinosporean stages of parasites of the phylum Myxozoa. Diseases of Aquatic Organsims 30: 1-9.

MACKINNON, D.L. & ADAM, D.I. 1924. Notes on Sporozoa parasitic in Tubifex. I. The life- history of *Triaetinomyxon* Štolc. Quarterly Journal of Microseopical Science 68: 187-209.

MARQUÈS, A. 1984. Contribution à la connaissance des Actinomyxidies: ultrastructure, cycle biologique, systématique. Ph.D. thesis, Université des Sciences et Techniques du Languedoc, Montpellier, France.

MOLNÁR, K., EL-MANSY, A. SZÉKELY, CS. & BASKA, F. 1999a. Development of *Myxobolus dispar* (Myxosporea: Myxoholidae) in an oligochaete alternate host, *Tubifex tubifex*. Folia Parasitologica 46: 15-21.

1999b. Experimental identification of the actinosporean stage of *Sphaerospora renicola* Dykova & Lom 1982 (Myxosporea: Sphaerosporidae) in oligochaete alternate hosts. Journal of Fish Diseases 22: 143-153.

ÖZER, A. & WOOTTEN, R. 2000. The life cycle of *Sphaerospora truttae* (Myxozoa: Myxosporea) and some features of the biology of both the actinosporean and myxosporean stages. Diseases of Aquatic Organisms 40: 33-39.

PALMER, R. 1995. Abstract. *Kudoa* in Ireland. European Association of Fish Pathologists Seventh International Conference. European Association of Fish Pathologists, England.

PERKINS, F.O. 1990. Phylum Haplosporidia. Pp. 19-29. In Margolis, L., Corliss, J.O., Melkonian, M. & Chapman, D.J. (eds) Handbook of Protoctista. (Jones & Bartlett: Boston).

POINAR, J.R., G.O. 1976. Presence of Mermithidae (Nematoda) in invertebrate paratenic hosts. Journal of Parasitology 62: 843-844.

1978. Associations between nematodes (Nematoda) and oligochaetes (Annelida). Proceedings of the Helminthological Society of Washington 45: 202-210.

RADULESCU, I. & MOTILICA, I. 1957. Contributti la cunoasterca parazitilor Oligochetelor din Marea Neagra: *Sphaeractinomyxon stolci* Caullery-Mesnil, 1904, colectat din *Tubifex* sp. Academia Republicii Populare Romine 7: 953-956.

RAFTOS, D.A. & COOPER, E.L. 1990. Diseases of Annelida. Pp. 229-243. In Kinne, O. (ed.) Diseases of marine animals, 3. (Biologische Anstalt Helgoland: Hamburg).

ROUBAL, F.R., HALLETT, S.L. & LESTER, R.J.G. 1997. First record of triactinomyxon actinosporean in marine oligochaete. Bulletin of the European Association of Fish Pathologists 17: 83-85.

SMITH, M.E. 1985. Naididae (Oligochaeta) as hosts for mermithid nematodes (Enoplida: Mermithidae). Canadian Journal of Zoology 63: 1459-1462.

SZÉKELY, C.S., MOLNÁR, K., ESZTERBAUER, E. & BASKA, F. 1999. Experimental detection of the actinospores of *Myxobolus pseudodispar* (Myxosporea: Myxobolidae) in oligochaete alternate hosts. Diseases of Aquatic Organisms 38: 219-224.

USPENSKAYA, A.V. 1995. Alternation of actinosporean and myxosporean phases in the life cycle of Zschokkella nova (Myxozoa). Journal of Eukaryotic Microhiology 42: 665-668.

WEISER, J. 1985. Phylum Myxozoa Grassé, 1970. Pp. 384-392. In Lee, J.J., Hutner, S.H. & Bovee, E.L. (eds) Illustrated guide to the Protozoa. (Society of Protozoologists: Kansas).

WOLF, K., MARKIW, M.E. & HILTUNEN, J.K. 1986. Salmonid whirling disease: *Tubifex tubifex* (Müller) identified as the essential oligochaete in the protozoan life cycle. Journal of Fish Diseases 9: 83-85.

XIAO, C. & DESSER, S.S. 1998a. Actinosporean stages of Myxozoan parasites of oligochaetes from Lake Sasajewun, Algonquin Park, Ontario: new forms of triactinomyxon and raabeia. Journal of Parasitology 84: 998-1009.

1998b. Actinosporean stages of Myxozoan parasites of oligochaetes from Lake Sasajewun, Algonquin Park, Ontario: new forms of echinactomyxon, neoactinomyxum, aurantiactinomyxon, guyenotia, synactinomyxon and antonactinomyxon. Journal of Parasitology 84: 1010-1019.

1998c. The oligochaetes and their actinosporean parasites in Lake Sasajewun, Algonquin Park, Ontario. Journal of Parasitology 84: 1010-1019.

YOKOYAMA, H., OGAWA, K. & WAKABAYASHI, H. 1991. A new collection method of actinosporeans - a probable infective stage of myxosporeans to fishes - from tubificids and experimental infection of goldfish with the actinosporean, *Raabeia* sp. Gyobyo Kenkyu 26: 133-138.

1993a. Involvement of *Branchiura sowerbyi* (Oligochaeta: Annelida) in the transmission of *Hoferellus carassii* (Myxosporea: Myxozoa), the causative agent of kidney enlargement disease (KED) of goldfish *Carassius auratus*. Gyobyo Kenkyu 28: 135-139.

1993b. Some biological characteristics of actinosporeans from the oligochacte *Branchiura sowerbyi*. Diseases of Aquatic Organisms 17:223-228.